Utility of Interferon-γ Release Assay Results to Monitor Anti-Tubercular Treatment in Adults and Children

Elena Chiappini, MD, PhD, Francesca Fossi, MD, Francesca Bonsignori, MD, Sara Sollai, MD, Luisa Galli, MD, and Maurizio de Martino, MD

Department of Science for Woman and Child’s Health, Meyer Children’s Hospital, University of Florence, Florence, Italy

ABSTRACT

Background: Interferon-γ release assays (IGRAs), including the commercially available T-SPOT.TB, Quantiferon-TB Gold (QFT-G), and Quantiferon-TB Gold In-Tube (QFT-G-IT), enable detection of circulating T lymphocytes responsive to specific Mycobacterium tuberculosis antigens. Studies of the potential role of serial IGRAs for assessment of response to anti-tubercular therapy are accumulating.

Objective: The objective of this systematic review was to evaluate the potential clinical utility of serial IGRAs in anti-tubercular therapy.

Methods: We conducted a literature search of the Cochrane Library and MEDLINE by PubMed, from database inception through October 1, 2011, for serial IGRA results in anti-tubercular therapy, in adults and children, using commercial standardized assays. All types of articles in the English language were included. Meta-analysis was performed to estimate the pooled percentage of reversion from a positive to a negative IGRA value at 3- to 6-month follow-up.

Results: According to inclusion and exclusion criteria, three T-SPOT.TB–based (n = 319 patients), three QFT-G–based (n = 75 patients), and seven QFT-G-IT–based (n = 558 patients) longitudinal studies were included. The percentage of patients with reversion from a positive to a negative IGRA value ranged from 5.71% to 13.93% for T-SPOT.TB, 5.26% to 71.05% for QFT-G, and 14.28% to 41.89% for QFT-G-IT assays. Meta-analysis estimation of reversion was feasible only for the QFT-G-IT assay, at 30.54% (95% CI, 22.89–38.75). In two pediatric studies, which were QFT-G-IT based (n = 122 children), the reported reversion rates were 14.28% and 20.33%, respectively.

Conclusions: Because IGRAs require time and cost resources, and reversion from positive to negative IGRA values occurs in a minority of treated patients, monitoring IGRA changes over time seems to have only speculative value in adults. Data in children are poor, but are in line with results reported in adults. (Clin Ther. 2012;34:1041–1048) © 2012 Elsevier HS Journals, Inc. All rights reserved.

INTRODUCTION

The availability of interferon-γ (IFN-γ) release assays (IGRAs) offers new possibilities for the diagnosis of tuberculosis (TB). IGRAs including the commercially available assays T-SPOT.TB (T-SPOT; Oxford Immunotec, Ltd., Abingdon, Oxfordshire, England), and Quantiferon-TB Gold (QFT-G) and Quantiferon-TB Gold In-Tube (QFT-G-IT) (both from Cellestis, Ltd., Carnegie, Victoria, Australia), enable detection of circulating T cells responsive to specific Mycobacterium tuberculosis antigens. False-positive results described with the use of the tuberculin skin test due to previous Bacille Calmette Guérin (BCG) vaccination or to infections with nontuberculous mycobacteria are thought to be averted or minimized using IGRAs.1–4 Accordingly, higher specificity of IGRA with respect to the tuberculin skin test has been reported in adults and children.5–8 Moreover, in contrast to the tuberculin skin test, IGRAs can be repeated without concerns about sensitization and boosting, and only one visit is required.

Emerging data suggest a potential role for IGRAs in monitoring the response to anti-tubercular therapy.1–16 IGRAs rely on a short period of incubation (~24 hours), and detect the response of activated effector T lymphocytes rapidly releasing IFN-γ after stimulation with specific antigen.1 Conversely, central
memory T cells produce effector cytokines within several days. Thus, a positive IGRA result should be due to a present infection and not to a remote memory. Because the effector response is driven by the antigen load, it has been suggested that the number of effector T lymphocytes is increased in the presence of active mycobacterial replication such as active disease. Successful treatment is associated with a reduced antigen load and, subsequently, with a lower number of effector cells specific to M. tuberculosis antigen, leading to a reduction in IFN-γ production and possibly resulting in reversion of the IGRA result from positive to negative.

Studies of the significance and interpretation of IGRA results after anti-tubercular therapy are rapidly accumulating. To investigate the potential clinical utility of serial IGRA in assessing the response to treatment, we reviewed the available literature.

METHODS
Definition of Reversion
Reversion was defined as the presence of a positive baseline IGRA value and a negative follow-up IGRA result, according to the manufacturer’s adopted cutoffs, as previously described.

Search Strategy
We performed a literature search of the Cochrane Library and MEDLINE by PubMed, from database inception through October 1, 2011, using the following terms: IGRA[Title/Abstract] OR (Interferon-gamma[Title/Abstract] AND release[Title/Abstract] AND assay[Title/Abstract]) OR T-SPOT.TB[Title/Abstract] OR QuantiFERON[Title/Abstract] AND (follow-[Title/Abstract] AND up[Title/Abstract]) OR reversion[Title/Abstract] OR longitudinal[Title/Abstract]) AND (Humans[MeSH Terms] AND English[lang]). Limits were field [Title/Abstract], species [Humans], and language [English]. A similar search was also conducted of the EMBASE database, which yielded no additional pertinent studies. Bibliographies in all relevant articles were then evaluated, and pertinent articles were included.

Study Selection
After the primary search was conducted by reviewing titles and abstracts of studies, the full texts of eligible studies were screened for inclusion, and study quality was assessed using the MOOSE (Meta-analysis of Observational Studies in Epidemiology) checklist. Relevant data were extracted and entered into tables. Study eligibility, quality assessment, and data extraction were checked for validity by a second author. An article was included in this review if it contained original data from adults or children or longitudinally evaluated changes in IFN-γ levels by using a commercial IGRA after 3 to 6 months of anti-tubercular therapy in latent TB infection (LTBI) or active TB.

All patients with active TB should have received at least the classic 6-month course of therapy, starting with three or four classic anti-tubercular drugs (rifampicin, isoniazid, pyrazinamide, and/or ethambutol). According to disease severity and risk of resistance, other anti-tubercular therapies including second- or third-line drugs could have been used, as judged by the treating physician. Patients were classified as responders to treatment if after 6 months of specific anti-tubercular therapy the results of microbiologic cultures in appropriate specimens were negative and there was clinical and radiologic improvement, as determined by findings at chest radiography or computed tomography. All patients with LTBI should have received isoniazide for 9 months or isoniazide plus rifampicin for 3 months. Compliance should have been assessed during follow-up, or directly observed therapy should have been administered.

Studies of serial IGRA performed in individuals exposed to or infected with TB who were not receiving anti-tubercular therapy were excluded, as were cross-sectional studies. Studies that originated from the same center were checked to ensure that the same data set was not replicated.

Statistical Analysis
For each selected study, the percentage of patients with reversion of IGRA values was reported, and 95% CIs were calculated. We attempted to perform a formal meta-analysis and calculate pooled estimates for the percentage of IGRA reversion from a positive to a negative value after 3 to 6 months of anti-tubercular therapy. Preliminary analyses showed that data from only three studies using QFT-G and another three studies using T.SPOT.TB were eligible (Figure 1), not allowing data from these assays to be pooled. Therefore, only data from eligible studies using QFT-G-IT were pooled in a formal meta-analysis. The percentages of reversion of IGRA values were pooled using the Der Simonian and Laird method using a random effects model to
account for heterogeneity between studies.\textsuperscript{18,19} The Cochran Q test was used to assess homogeneity between studies.\textsuperscript{19} The magnitude of heterogeneity between studies was expressed as the variance percentage attributable to between-studies heterogeneity, the I\textsuperscript{2} statistic.\textsuperscript{19} Statistical analyses were performed using StatsDirect software (www.statsdirect.com).

**RESULTS**

One hundred seventy-four articles were initially identified, of which 29 studies were initially selected for relevance and pertinence, according to the search strategy (Figure 1 and Table). At further assessment, two cross-sectional studies\textsuperscript{20,21} and one study that did not separately provide data from treated and untreated patients\textsuperscript{10} were excluded.

**Studies Using T-SPOT.TB**

Six studies using T-SPOT.TB were initially identified.\textsuperscript{7,9,10,16,20,21} (Table). However, two studies including, respectively, 33 and 79 adults with active TB, had major limitations: longitudinal data from the same patient were not available,\textsuperscript{20} and results obtained compared data from patients at the beginning of treatment with data from other patients still receiving or at the end of treatment.\textsuperscript{21} In both of these studies, a lower percentage of negative T-SPOT.TB results was observed in treated versus untreated patients (83\% vs 19\%,\textsuperscript{20} and 83.3\% vs 69.8\%\textsuperscript{21}; however, these data should be interpreted with caution because of the cross-sectional study design. In another study, a high variability in IGRA pattern response in 122 adults with LTBI tested using both QFT-G-IT and T-SPOT.TB was reported, with a strong concordance between the two tests ($\kappa = 0.7–0.8$).\textsuperscript{10} The overall percentage of individuals with a positive result remained stable at approximately 45\% to 50\%. A dynamic pattern of response with reversions or conversions occurred more often when T.SPOT-TB or QFT-G-IT results were intermediate (5 to 50 spots or 0.25–4.0 IU/mL) than those observed in individuals with lower or higher values.\textsuperscript{10} Of note, only 29.5\% of these patients completed preventive isoniazide therapy, and data from treated and untreated patients could not be derived separately.\textsuperscript{10}

The percentage of patients with reversion at 6 months was 5.71\% in one study from Switzerland that included 35 adults with active TB and positive T-SPOT.TB results at baseline,\textsuperscript{9} and 10\% in a study from the United States that included 40 adults with active TB.\textsuperscript{7} T-SPOT.TB and QFT-G-IT were simultaneously used in another study that included 275 patients with active TB. The percentage of patients with reversion was higher for QFT-G-IT than for T-SPOT.TB (39.2\% vs 13.9\%).\textsuperscript{16}
Studies Using QFT-G

The QFT-G assay was the first commercially available ELISA-based assay, and subsequently was modified as the QFT-G-IT. Three studies using QFT-G were identified,8,11,22 including a total of 75 patients with a positive result at baseline (Table).

In a study from Italy,11 a 70% reduction in IFN-γ levels at 6 months (from 17.5 [2.7] IU/mL to 1.3 IU/mL; P < 0.0001) was reported in 19 patients with TB; however, in only one patient (5.1%) did the value revert from positive to negative. In another study from Italy22 that included 38 patients with active TB, IFN-γ levels significantly decreased (from a median value of 2.79 UI/mL to 0.02 UI/mL; P < 0.001) at 6 months of therapy in 27 patients with reversion of IGRA values (71.1%) with clinical resolution, but remained stable.

Table. Characteristics of studies using a commercially available interferon-γ release assay (IGRA).

<table>
<thead>
<tr>
<th>Source, Year</th>
<th>Country</th>
<th>Population</th>
<th>No. (%)</th>
<th>95% CI</th>
<th>Follow-Up, Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patients With Reversion of IGRA Values</td>
<td></td>
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</tr>
<tr>
<td>T-SPOT.TB</td>
<td></td>
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</tr>
<tr>
<td>Ribeiro et al,2009</td>
<td>United States</td>
<td>Adults with active TB</td>
<td>4/40 (10.0)</td>
<td>2.79–26.66</td>
<td>6</td>
</tr>
<tr>
<td>Bozward et al,2009</td>
<td>Switzerland</td>
<td>Adults with active TB</td>
<td>2/35 (5.71)</td>
<td>0.69–19.15</td>
<td>6</td>
</tr>
<tr>
<td>Chee et al,2010</td>
<td>Singapore</td>
<td>Adults with culture-confirmed TB</td>
<td>34/244 (13.93)</td>
<td>9.84–18.92</td>
<td>6</td>
</tr>
<tr>
<td>Dominguez et al,2010</td>
<td>Spain</td>
<td>Adults with active TB; cross-sectional study</td>
<td>Longitudinal data for same patient were not available</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dheda et al,2007</td>
<td>Canada</td>
<td>Adults with culture-confirmed TB</td>
<td>Longitudinal data for the same patient available only for three patients</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Franken et al,2008</td>
<td>The Netherlands</td>
<td>TST-positive adults with TB contacts</td>
<td>Data from treated and untreated patients not derived separately</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>QuantiFERON-Gold-TB</td>
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<tr>
<td>Sauzullo et al,2009</td>
<td>Italy</td>
<td>Adults with active TB</td>
<td>27/38 (71.05)</td>
<td>54.09–84.57</td>
<td>6</td>
</tr>
<tr>
<td>Kobashi et al,2008</td>
<td>Japan</td>
<td>Adults with active TB</td>
<td>9/18 (50.0)</td>
<td>26.02–73.98</td>
<td>6</td>
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<tr>
<td>Goletti et al,2007</td>
<td>Italy</td>
<td>Adults with recent TB contact</td>
<td>1/19 (5.26)</td>
<td>0.13–26.02</td>
<td>6</td>
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<tr>
<td>QuantiFERON-Gold-TB In tube</td>
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<tr>
<td>Dyrholl-Rise et al,2010</td>
<td>Norway</td>
<td>Adults with LTBI</td>
<td>15/40 (37.50)</td>
<td>22.72–54.19</td>
<td>3</td>
</tr>
<tr>
<td>Lee SH et al,2010</td>
<td>South Korea</td>
<td>Adults with LTBI</td>
<td>31/74 (41.89)</td>
<td>30.51–53.93</td>
<td>4</td>
</tr>
<tr>
<td>Lee SW et al,2010</td>
<td>South Korea</td>
<td>Adults with active TB</td>
<td>18/57 (31.57)</td>
<td>19.90–45.24</td>
<td>6</td>
</tr>
<tr>
<td>Bocchino et al,2010</td>
<td>Italy</td>
<td>Adults with culture-confirmed TB</td>
<td>15/53 (28.30)</td>
<td>16.78–42.34</td>
<td>6</td>
</tr>
<tr>
<td>Hermann et al,2009</td>
<td>France</td>
<td>Children with LTBI or active TB</td>
<td>9/63 (14.28)</td>
<td>6.74–25.39</td>
<td>3</td>
</tr>
<tr>
<td>Nenadic et al,2011</td>
<td>Croatia</td>
<td>Children with LTBI or active TB</td>
<td>12/59 (20.33)</td>
<td>10.97–32.83</td>
<td>6</td>
</tr>
<tr>
<td>Chee et al,2010</td>
<td>Singapore</td>
<td>Adults with culture-confirmed TB</td>
<td>83/212 (39.15)</td>
<td>32.54–46.07</td>
<td>6</td>
</tr>
<tr>
<td>Dominguez et al,2010</td>
<td>Spain</td>
<td>Adults with active TB; cross-sectional study</td>
<td>Longitudinal data for same patient not available</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Franken et al,2008</td>
<td>The Netherlands</td>
<td>TST-positive adult with TB contacts</td>
<td>Data from treated and untreated patients not provided separately</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, data not available; TB, tuberculosis; LTBI, latent tuberculosis infection.
Studies Using QFT-G-IT

Nine studies were initially identified using QFT-G-IT. However, data for treated patients were not separately provided in one, and one study was cross-sectional. Thus, seven studies were finally included. In a study from Croatia, 35 of 40 patients (87.5%–84.6%) still had positive results of QFT-G-IT after 3 to 15 months of therapy. IFN-γ levels were comparable at the start of the study (mean [SD] at baseline, 4.13 [3.99] IU/mL) and after 3 and 15 months (5.65 [4.14] IU/mL), indicating that IGRA should not be used as a reliable marker for monitoring the efficacy of therapy. A decrease in IFN-γ levels was reported in 97.3% of 214 Korean subjects. The authors of that study suggested that IGRA may be a useful tool for evaluating therapeutic response to rifampicin prophylaxis; however, reversion of test results occurred in only 41.9% of subjects. In another large study from Singapore in patients with active TB, a substantial percentage of patients had positive results of IGRA after treatment of TB, and the reversion rate was 39.2% (83 of 212 patients) with QFT-G-IT, and 13.9% (34 of 244) with T.Spot-TB. The kinetics of the quantitative T-cell responses was not significantly different between patients with or without risk factors for disease relapse.

Studies In Children

Commercial IGRA (QFT-G-IT) have been used in two pediatric studies, overall including 122 children. In one study, children with LTBI and active TB experienced a similar and significant rebound at day 10 of treatment, with higher IFN-γ values than those at baseline ($P = 0.035$). A subsequent decline in IFN-γ response was observed at days 0 and 90 or 180 ($P = 0.942$); however, reversion occurred only in a minority of patients, 9 of 63 (14.3%). In a study of 59 Croatian children aged 4 to 18 years, investigated before and after completion of anti-tubercular therapy, no difference was found in IFN-γ concentration between children with LTBI or active TB, neither before nor after treatment. Sustained IFN-γ levels persisted for at least 2 years after completion of treatment. Considered together, these findings suggest that it is not possible to categorize responders and non-responders to therapy according to the presence or absence of reversion of QFT-G results.

Results of Meta-Analyses

Based on the results from seven QFT-G-IT studies, overall including 558 patients with TB with positive IGRA results at baseline, a meta-analysis estimate of the pooled percentage of patients with reversion of IGRA values after 3 to 6 months of anti-tubercular treatment for QFT-G-IT was calculated (Table). This pooled estimated percentage of reversion was 30.54% (95% CI, 22.89–38.75) for QFT-G-IT (Figure 2). Inconsistency ($I^2$) was 70.8% (95% CI, 31.2%–86.3%).

DISCUSSION

To our knowledge, this is the first systematic review to explore the available data regarding changes in IGRA response during anti-tubercular therapy in adults and children. A significant decrease in IFN-γ production during anti-tubercular therapy have been reported in several studies. However, even when present, variations in IFN-γ levels may vary largely between individuals, and reversion to negative values occurred only in a minority of patients. For QFT-G-IT and T-SPOT.TB, the rate of reversion ranged from 14% to 42% and from 6% to 14%, respectively. Several factors may explain the low reversion rates observed in our review. Reinfection with *M. tuberculosis*, in particular in high-burden settings, or continuous exposure to environmental mycobacteria expressing ESAT-6 or CFP-10 homologues is a possible explanation for the persistent IGRA positivity despite effective therapy. Alternatively, it is possible that circulating effector T cells may persist for a long time after clearance of the infection. In addition, several factors may interfere with the IGRA response, including endotoxin contamination, ethnic and nutritional characteristics of the patients, and HIV or helminth co-infection. An intriguing possibility is that, at least in a subgroup of patients without reversion, persistent IGRA positivity is due to lack of sterilization, and it
may be a hallmark of failure of therapy. Encouraging results in this regard were described in 2004 in a small study from Italy based on an in-house ELISPOT (ELISA spot assay) that demonstrated reversion in patients with clinical response to anti-tubercular therapy but not in those exhibiting clinical failure. However, subsequent large studies failed to demonstrate different percentages of reversion between patients with or without clinical response to treatment.

The biological explanation for reversion is unclear. Some authors have speculated that reversion may be due not only to a reduction in IFN-γ-producing antigen-specific effector T cells during effective treatment, but that reversion may also reflect biological fluctuations in IFN-γ production or variability in laboratory procedures. In addition, they may be influenced by the life cycle of M. tuberculosis, with phases of active replication and of the dormant state, associated with variations in the amount of secreted antigens such as ESAT-6 and CFP-10. Inasmuch as IGRA results require time and cost resources, and interpretation of minimal variation in the IFN-γ level does not lead to change in clinical management of patients, based on our results, monitoring of IGRA changes over time seems to have only speculative value in adults.

Data in children are limited. It has been reported that IGRA performance may vary largely between adults and children. For example, higher percentages of indeterminate results in children compared with adults have been reported by some authors but not confirmed by others. IGRA results may be influenced by the child’s age and immunologic status, including both physiologic immaturity and primary or acquired immunodeficiency. The two pediatric studies selected in this review, both of which used the QFT-G-IT assay, reported a reversion rate of 14.3% and 20.3%, which suggests that in children, as in adults, serial IGRA results are not reliable for monitoring response to therapy. The first rebound might confirm the potential of IGRA to detect recently acquired infection. Anti-tubercular therapy may restore active T-cell response, as noted in vivo in the TB paradoxic reaction, leading to improvement in IGRA results. However, caution must be exercised in interpreting these changes, as they may not necessarily imply a clinical response.

**Figure 2.** Forest plot shows percentage of patients with reversion in longitudinal studies using one of the commercially available interferon-γ release assays (IGRAs): T-SPOT.TB, QuantiFERON-Gold, and QuantiFERON-Gold In-Tube. Squares and horizontal lines correspond to the recorded percentage of patients with reversion of IGRA values at 3-to 6-month follow-up in patients with tuberculosis with positive IGRA results at baseline, and their respective 95% CIs. Squares reflect the weight each study contributed to the analysis. The diamond represents the pooled value, and correspondent 95% CI. n/N, number of patients with reversion and total number of patients with a positive IGRA value at baseline.
to sustained IFN-γ production even in the presence of a lower bacterial burden.\textsuperscript{2}

The present review has several limits. Our search strategy may have resulted in some studies being missed because the decision to include only studies published in English may have excluded some data. In addition, we decided to include only data from studies based on commercial standardized IGRA\textsuperscript{s}, excluding results from 13 articles using an in-house ELISPOT assay (data not shown). Methods and cutoffs in these studies were heterogeneous. Increased or persistently positive IFN-γ levels during and after treatment have been reported by some authors\textsuperscript{27} but not confirmed by others.\textsuperscript{12,23,28} Again, the clinical utility of such decay as a surrogate marker of treatment efficacy has been questioned because in half of the pediatric\textsuperscript{3} and adult\textsuperscript{5} patients, ELISPOT results remained positive at the completion of treatment of TB. Together, these findings are in line with those reported in our systematic review of studies using commercial IGRA\textsuperscript{s}.

Another possible limitation of our study is that results in patients with LTBI were not differentiated from results in those with active TB because only three studies directly compared these two populations.\textsuperscript{1,2,15} In particular, one study in adults and two studies in children found no difference in IFN-γ dynamics between patients with LTBI or active TB.\textsuperscript{1} Finally, the present review did not examine other interesting issues that may arise from studies of serial IGRA\textsuperscript{s} such as the frequency and possible significance of conversion from a negative to a positive value, the IGRA predictive value for developing TB in exposed subjects, and the possible significance of the different dynamics of response to ESAT-6 and CFP-10 antigens. Each of these issues deserves a separate in-depth study.

CONCLUSIONS
Available data are now sufficient to suggest that, from a practical point of view, monitoring changes in IGRA response during anti-tubercular treatment is of limited usefulness in adults. Data in children are poor, but are in line with results reported in adults.

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CONFLICTS OF INTEREST
The authors have indicated that they have no conflicts of interest regarding the content of this article.

REFERENCES

**Address correspondence to:** Elena Chiappini, Department of Science for Woman and Child’s Health, Meyer Children’s Hospital, University of Florence, Viale Pieraccini, 24, 50100, Florence, Italy. E-mail: elena.chiappini@unifi.it